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Award Number: DAMD17-02-1-0629

TITLE: Structural Basis for the Pharmacological Rescue of Mutant p53 with Small Molecule Compounds

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REPORT DATE: April 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2004	Annual Summary (1 Apr 2003 - 31 Mar 2004)

4. TITLE AND SUBTITLE

Structural Basis for the Pharmacological Rescue of Mutant p53 with Small Molecule Compounds

5. FUNDING NUMBERS

DAMD17-02-1-0629

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The p53 protein is a tumor suppressor crucial to maintaining genomic integrity. In the event of DNA damage, p53 is responsible for transcribing genes leading to cell death. A class of mutations which occur in the core domain (102-292) leads to thermodynamic destabilization and inability to bind its cognate DNA sequence. Small molecules which bind to and stabilize mutant p53 core domain have potential to be therapeutically useful. Two potential "hot spots" on the surface of the mouse p53 core domain have been discovered which can be targeted by small molecule compounds. One hot spot was discovered by soaking the crystal lattice with various organic solvents, and locating the solvents in the electron density. Another potential hot spot was located in the high resolution structure of the mouse core domain where a molecule of tris(hydroxymethyl)aminomethane (Tris) was observed to bind on the surface of the protein, making numerous hydrogen bonding contacts. The design and synthesis of molecules which bind to these areas is currently ongoing.

14. SUBJECT TERMS

Not Provided

15. NUMBER OF PAGES

11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

The p53 tumor suppressor protein is crucial to maintaining genomic integrity. In the event of DNA damage, p53 activates transcription of genes which lead to apoptosis or cell cycle arrest[1]. As many as 50% of all human cancers are associated with mutations to p53[2]. The p53 protein has four domains: an amino terminal transactivation domain (residues 1-44), a core DNA binding domain (102-292), a tetramerization domain (residues 320-356), and a carboxy-terminal regulatory domain (residues 320-356). An estimated 95% of all tumorogenic mutations to the p53 gene occur within the core domain[3]. Most of these mutations to the core domain occur as point mutations, which can be generally classified into two groups: 1) those which occur to amino acids making direct contact with DNA, thereby decreasing binding affinity, and 2) those which cause the core domain to be unstable, causing unfolding or misfolding, and therefore inability to bind DNA. To address mutations which cause the core domain to be unstable, attempts have been made to introduce small molecules which have the capability to bind to and stabilize the core domain, rescuing function. Among these attempts are those made by Freidler and co-workers[4], who have designed a peptide with micromolar affinity and the ability to rescue human p53 core domain function. Knowledge of areas on the p53 core domain surface which can potentially be targeted by designed small molecules can be helpful.

Body

Since the last update provided to the Department of Defense, work has progressed on several fronts.

1.) ***Structurally characterize the binding of peptides that have been shown to interact with and stabilize the p53 core domain using x-ray crystallography.*** Work was attempted to structurally characterize the binding of peptides described by Fersht et al. that has been shown to bind to and stabilize the p53 core domain[4; 5]. To that end, the human core domain was purified and co-crystalization was attempted by mixing the peptides and the core domain. Potential crystallization conditions were then screened. Various crystal forms were observed, and refined. Best crystals were taken to the Advanced Photon Source (APS) at Argonne National Laboratories (ANL). Crystals formed in the spacegroup $P2_12_12$ and diffracted to a maximum of 2.2Å. After refinement and close examination of the electron density, no evidence of the peptide was observed. Other groups have also tried a similar approach. Likewise, they have not seen any peptide in the crystal lattice[6].

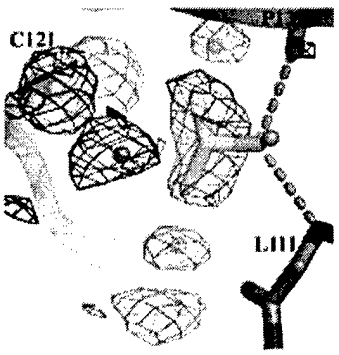


Figure 1: Density surrounding the location of the isopropanol binding site. Blue density-$Fo_{(isopropanol)}$-$Fo_{(native)}$ at 2.5ó level ; Red density-$Fo_{(native)}$-$Fo_{(isopropanol)}$ at 2.5ó level; blue spheres-water molecules observed in native structure; green sphere-water observed in isopropanol soaked structure; orange dashes-hydrogen bonding.

2.) ***Soak high resolution crystals of the mouse p53 core domain with organic solvents in order to locate possible binding sites for small molecules.*** Crystals of the mouse p53 core domain were soaked with 1,6 hexanediol, acetonitrile, isopropanol, acetone, and phenol. Typically, the crystals were frozen in ~35% solvent and then frozen. Data collection then proceeded

and the solvent molecules were located by viewing f_o-f_c, f_o-f_o and $2f_o$-f_c electron density. Of all solvents tried, one isopropanol molecule was located in the electron density, and was observed to displace several water molecules observed in the native structure (figure 1). Furthermore, the hydroxyl group of the isopropanol molecule was observed to be bridging two â-sheets, with its methylene groups pointing to a hydrophobic region (figure 2). This area is currently being investigated as a potential binding site for the introduction of small molecules that have the possibility to interact with the p53 core domain. Several molecules were designed and tested based on the observed binding mode of the isopropanol, but none have been shown any affinity thus far.

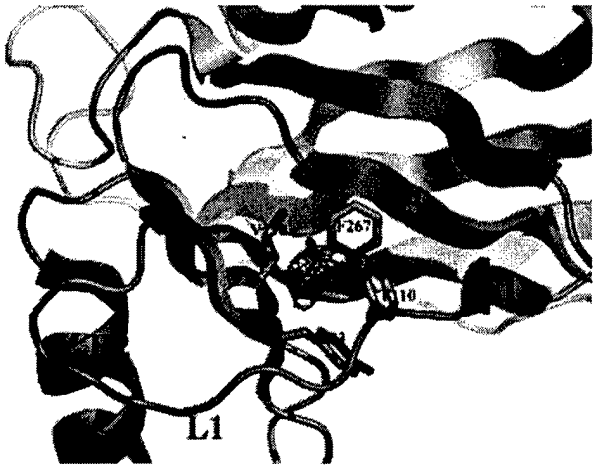


Figure 2: Fo-Fc omit map calculated at 2.5ó using the final structure with isopropanol omitted.

3.) ***Refinement of the high resolution structure of the mouse p53 core domain.*** The high resolution structure (1.55 Å) of the mouse p53 core domain was refined further to prepare for publication. During the course of the refinement, continuous electron density was observed which could not be explained. Finally, a molecule of tris(hydroxymethyl)aminomethane (Tris) was built into the electron density and refined for several cycles. Tris was present in the mother liquor, and in our crystal structure, it is observed forming hydrogen bonding interactions with various surface residues. The Tris molecule binds between the S1 and S10 strands of the protein and makes numerous direct

hydrogen bonding interactions with the side chain atoms of residues Y126, Q128, and D265 (figure 3). Additionally, the Tris molecule makes a water-mediated hydrogen bonding interactions to the backbone carbonyl of S266 and to the side chain nitrogen of Q101. It is not unprecedented to see Tris bound to active sites of proteins[7], and its presence in the p53 crystals could be a lead to design further molecules with increased affinity. To that end, molecule **1** was designed and synthesized as a potential molecule with increased affinity towards the p53 core domain. Unfortunately, **1** did not show any affinity to the mouse p53 core domain. At this time, further experiments are being performed to probe this site.

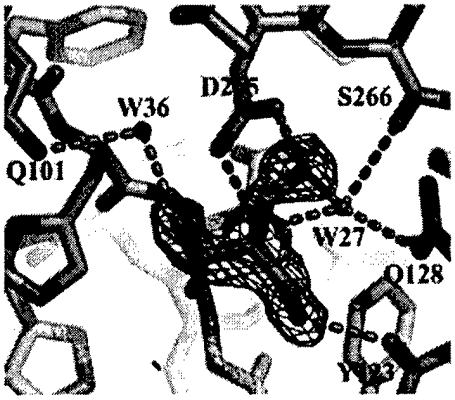
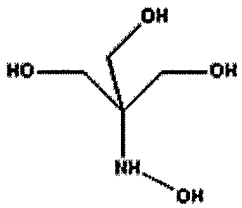


Figure 3: F_o-F_c omit density of Tris bound to the p53 core domain contoured at 2.06. Hydrogen bonding interactions are depicted in orange. Two molecules of water are depicted in red.



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Key research accomplishments

- Grew potential co-crystals of human p53 core domain/stabilizing peptides which diffracted to a maximum of 2.2Å.
- Collected data at the Advanced Photon Source of potential p53 core domain/stabilizing peptides, solved, and refined the structure. Determined that there was no peptide in the crystals.
- Soaked crystals of the mouse p53 core domain with various organic solvents including 1,6 hexanediol, acetonitrile, isopropanol, acetone, and phenol, and collected data. Typical crystals diffracted to ~2.0Å at our home source.
- Solved, and refined structures of crystals that have been soaked with organic solvents. A molecule was isopropanol was detected after examination of electron density.
- Further refined the high resolution (1.55 Å) structure of the mouse p53 core domain and built in a molecule of tris(hydroxymethyl)aminomethane (Tris).
- Designed and synthesized a molecule (**1**) with potential to bind the mouse p53 core domain. Binding was tested, and not detected.

Reportable outcomes

"High resolution crystal structure of the mouse p53 core domain: An analysis of protein flexibility and a framework for structure-based drug design" Ho, W.C., Zhao, K., Chai, X., and Marmorstein, R.M., *manuscript submitted.*

Conclusions

Several conclusions can be reached from this funded work. First, due to observations of lattice packing in human p53 core domain crystals that did not contain the stabilizing peptide, we believe that crystal packing interactions excluded the peptide from the lattice. In order to obtain a co-crystal, other approaches may have to be employed that prevent such exclusion from the crystal lattice, for example, cross linking. Second, we believe that we have located two "hot spots" on the surface of the mouse p53 core domain, and demonstrated two small molecules, isopropanol, and tris(hydroxymethyl)aminomethane (Tris) bound to the respective "hot spots", although binding affinity is weak. This information can be useful in designing molecules which bind with higher affinity. These findings have been written up and submitted for publication.

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